

## DIGESTIBILITY AND SAFETY OF LIMED HIDE COLLAGEN IN RAT FEEDING EXPERIMENTS

### INTRODUCTION

CONSIDERABLE QUANTITIES of collagen are available each year in the form of limed cattlehide parts unsuited to the manufacture of leather. Collagen obtained from the corium layer of split hides after neutralization and washing (Mellon and Korn, 1956; Deasy, 1959), is essentially pure except for inherent fat and ash. This protein has unusual chemical and physical properties including a unique fibrous structure not found in other natural proteins. The fact that collagen can be dispersed under a variety of conditions to produce preparations ranging from a stiff fibrous paste to a liquid virtually free of fibers, or to a gel which resists hot water (Whitmore et al., 1972), suggests that its binding and texturizing

properties may have uses in food or feed. Possible applications of these properties are now being investigated in the food and feed industries. At present, commercial use is limited to the manufacture of sausage casings which consumes only about 0.5% of the potential supply. The development of new uses, while not dependent on the nutritional qualities of collagen, requires knowledge of those qualities as measured by standards normally applied to food ingredients. Collagen represents about one-third of the total body protein (Gustavson, 1956) and does appear in various meat cuts. The present report does not deal with this native collagen per se, but with previously limed cattlehide collagen. Although differences between limed and unlimed or native collagen do exist (Veis, 1967), inferences may be made which will reflect on the nutritive and digestive properties of the native carcass collagen. While the digestibility of collagen has been investi-

gated to some extent, partly because of its resistance to isolated proteolytic enzymes (Banga, 1965; Lamphiah, 1966), and, also, due to its resistance to enzymes under physiological conditions, native fibrous collagen has often been regarded as indigestible (Grassmann, 1966; Ryan and Woessner, 1971; Cassel and Kanagy, 1949). Its low nutritional value as a protein in the form of gelatin was reported by Chapman et al. (1959) and Rama Rao et al. (1964), among others, and referred to by Stainsby and Ward (1969). Except in investigations of gelatin and soluble collagen as allergens (Maurer, 1954 and others), no reports of in vivo studies for toxicity have been found in the literature. This study was undertaken to fill this need for additional information on the digestibility and toxicity of limed cattlehide collagen. This paper reports the results of experiments in which collagen was fed to rats as an adjunct to the complete diet.

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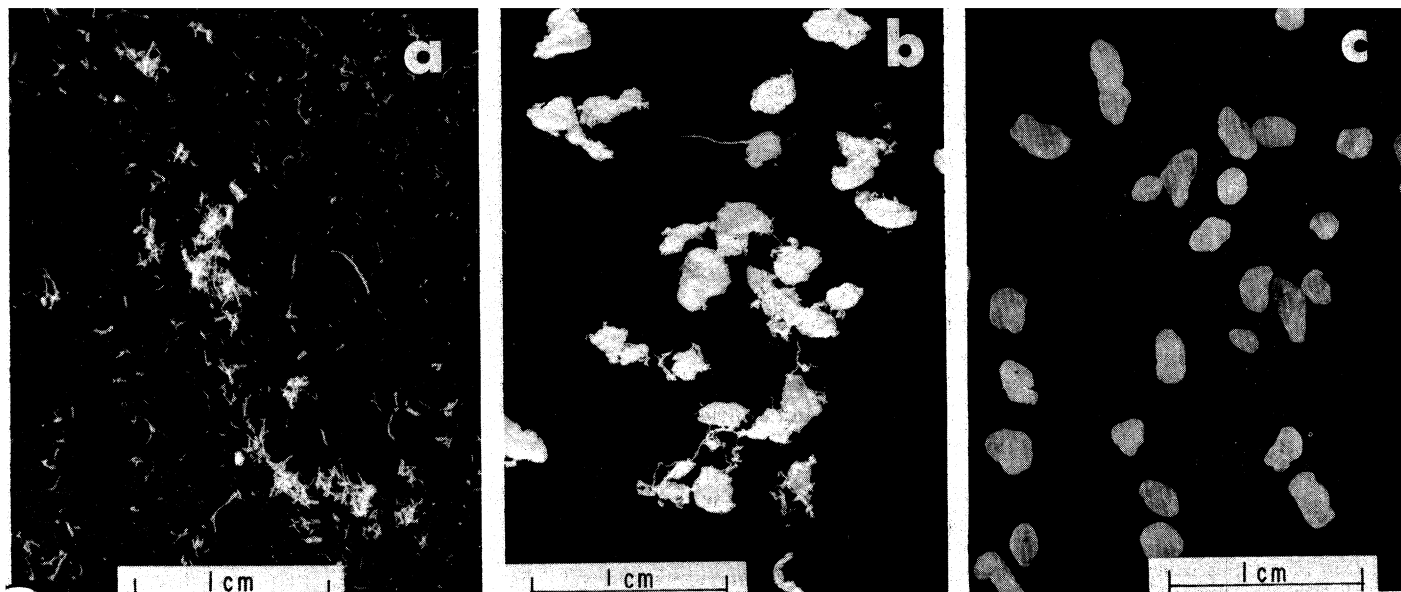


Fig. 1—Three types of collagen fed to rats. (a) Freeze-dried collagen, reground with dry ice in a cold Wiley mill to pass a 2 mm screen; (b) Particles produced by acetone dehydration of wet collagen cut through an 0.060 in. cutting head in an Urschel mill; (c) Particles produced by air-drying of wet collagen cut through an 0.060 in. cutting head in an Urschel mill. (Magnified 2.6X)

Table 1—Relative caloric assay on hide collagen vs. casein

Supplements added to purified basal diet <sup>a</sup>		Body weight changes (Avg per rat)		Moisture-free fecal wt/rat (g)
		in 7 days (g)	Net gain (g)	
None	0	+1.2	—	0.64
Casein	1.0	+10.8	+9.6	0.58
Collagen	1.0	+9.5	+8.3	0.62

<sup>a</sup>Each rat received 5g of purified basal diet per day, plus supplement indicated.

## EXPERIMENTAL

### Preparation and characteristics of collagen

Hide collagen from fresh limed cattlehide splits was prepared by a deliming, neutralizing treatment followed by thorough washing (Whitmore et al., 1970). The splits were then cut while frozen to obtain hide particles which passed a 10 mm perforated screen.

For rat feeding tests the particles were freeze dried in shallow pans resting on platens warmed to 57°C in a vacuum <3 cm Hg. The dehydration required about 8 hr and was repeated on several lots to make a single composite of about 15 kg. The lyophilized material was further ground dry in a cold Wiley mill with added dry ice to pass a 2 mm screen (Fig. 1a). This product was found to be 76.2% collagen based on Kjeldahl nitrogen, 10.8% moisture, 9.8% fat, 0.7% fiber and 0.4% ash. A water suspension had a pH of 5–6. No odor or taste was evident in this preparation. To estimate the gelatinization resulting from cutting, soluble material was eluted from 6g of 10 mm screened and lyophilized material with 40°C water at a rate of 200 ml/hr; 100 ml aliquots of extract were dried at 95°C and weighed. The three aliquots taken in the first hour accounted for about 87% of the solids extractable in 4 hr. The extracts in the last hour accounted for only 3% of the total extracted. The total solids extracted in 4 hr were found to be between 12.2 and 12.8% of the starting material on a dry basis. When the analysis was performed on material from the same source that was ground in the dry state to pass a 2 mm screen, the warm water extraction produced a 12.8% loss. This degradation from the cold dry cutting was not regarded as an important difference from the degradation resulting from cutting wet frozen material followed by lyophilization at 57°C.

For study of the effects of preparative techniques on caloric availability and digestibility, two other types of comminuted hide were prepared. Both were cut wet and cold (30% collagen dry basis) using an Urschel Comitrol fitted with a nominal 0.060 in. head (Elias et al., 1970). The resulting granules were 1–2 mm in largest dimension and quite easily separable. Only about 7% of the product was extracted with 40°C water under the conditions described above. One lot of this comminuted hide was extracted with 2 volumes of acetone for 1 hr, the extraction repeated four times, and dried slowly at 25°C while covered to prevent water condensation. The product was essentially fat free white granules (Fig. 1b). A second lot was dried in air by slowly tumbling in a stream of warm air. Some aggregation of granules oc-

curred and these were separated from the single granules by screening. The product was about 3% fat on a dry basis, smooth rounded light tan colored granules (Fig. 1c). These wet-cut granules, dried in the forms shown in Figure 1 and described above, were fed only as separate caloric availability and digestibility supplements.

### Determination of digestibility and caloric availability

To a basal diet calorically restricted and fed at near maintenance levels, described by Rice et al. (1957), collagen or casein were added as the only proteins. Rats were fed in separate cages 5g of basal diet plus 1g of supplement each day for 7 days. Body weights before and after the test period were used to compare caloric availability of the two proteins after adjustment for the weight changes in the controls. In further tests, the level of supplement was raised to 2g/rat/day. Acetone dried (defatted) collagen was compared with air dried (not defatted), and the effect of dry cutting and particle size on digestibility was tested. Fecal weights of rats fed control and supplemented diets were compared to estimate digestibility. Weight gains of the test animals were used to estimate caloric availability.

### Determination of toxicity

The collagen was mixed with a commercial rat diet at a level of 20%, fed ad lib to weanling (50g average) males and females. Casein added at the same level and the diet alone were used as controls. Groups of five rats of each sex were fed on each diet. During the 90-day feeding period, weekly body and feed consumption records were maintained. Urinalysis and hematology studies were made during the last 10 days of the test period. At the end of the feeding period body and organ weights were compared as were histological findings.

## RESULTS

### Digestibility and caloric availability

Table 1 shows results of a caloric availability test where the collagen supplement was added to a purified diet which was fed at a rate (5g/rat/day) to restrict caloric intake to a maintenance level. The weight gains shown by collagen-fed rats were 86% those of rats fed equal daily supplements of casein. Fecal weights also indicated the digestibility of the fibrous collagen at close to 100%.

In another test comparing caloric value

of the same collagen with that of gelatin (Nutritional Biochemicals Corp., Cleveland), the weight gains indicated that 1 g of collagen was equivalent to 1.5g of gelatin as an energy source.

In a subsequent test, 2g supplements per rat per day, 29% of the total diet, were fed (Table 2). A comparison was made of caloric availability between gelatin, 2 mm dry ground collagen (3), 0.060 in. acetone dried defatted (4), and 0.060 in. air dried collagen (5). (3, 4, 5 refer to Table 2 notation and to a, b, c in Fig. 1). Results at this level of supplementation show collagen 3 to be about 90% digestible and slightly lower in caloric availability than gelatin. Hide collagen cut wet and cold at 1–2 mm maximum dimension followed by acetone dehydration (4) or air drying (5) was compared to study the effect of fat or physical state on caloric availability. To minimize possible pseudo-weight gains due to undigestible intestinal residues, all rats were fed basal diet only (5g/rat/day) for a period of 48 hr following the 7-day period of supplementation. Fecal weights per se could not be obtained due to diarrhea, hence combined urine and feces weights were used. Since collagen (3) contained 10% fat, collagen (4) was essentially free of fat and collagen (5) contained about 3% on dry basis, the results indicate that within the range tested, fat content is not an important consideration in digestibility or caloric availability of fibrous collagen when it is fed to rats. It also appears that the degree of fiber development in a palatable or chewable size range does not affect digestibility in rats.

### Toxicity

Body weights and organ weights (Table 3) of rats fed the 2 mm dry ground collagen for 90 days were comparable to those of rats fed casein except for the kidney weights. In both sexes the kidneys were significantly heavier. The importance of this effect is minimized, since the microscopic examination of kidney tissue sections did not show any differences from comparable tissues of control animals. The results of the blood and urine analyses suggest that the only significant effect was an increase in hemoglobin concentration in the female rats fed casein and collagen. It was noted that during the collection of blood samples the rats fed collagen were more sensitive to ether anaesthesia, and the blood appeared to clot more rapidly. Pathological findings from studies of the stomach, small and large intestine, heart, trachea or larynx, lung, pancreas, liver, kidneys, urinary bladder, spleen, pituitary, thyroid (sometimes with parathyroid), adrenals, testes or ovaries, uterus in females (sometimes seminal vesicles in males), thymus and brain (sagittal section) indicated no significant lesions or differences between

the control and collagen-fed rats.

Footnote (b) to Table 2 notes that some rats in each group had diarrhea. Collagen and gelatin were fed at 2g/rat/day added to 5g basal diet in this test. This is at a rate of 28+% of the total diet, considerably higher than the 20% fed for 90 days for toxicity studies, and several times the level proposed for food texturizers. The diarrhea may be linked to the higher hemoglobin levels in the blood of some rats. This in turn may be linked to the susceptibility to ether anesthesia after 80 days on the diet. Since the diet was fed dry, an assumption was made that the water-holding capacity of the collagen might be simply dehydrating the animals. However, careful checks of water intake between test and control animals revealed

no significant differences. The cause and effect of these findings should be the basis for some future collagen feeding tests.

## DISCUSSION

### Digestibility

Studies of the digestibility of collagen by isolated enzymes have been reported in the literature. Mandl (1961) has reviewed the effects of temperature, acids, swelling, liming, grinding etc. on the susceptibility of collagen to attack by trypsin. Woessner (1968) has more recently reviewed the proteolytic digestion of collagen under physiological conditions of pH, temperature and ionic strength. Much of the literature is not relevant to our

findings because it is concerned with effects on soluble or reconstituted collagen rather than on native, insoluble or previously limed "matrix" collagen (Veis, 1967). The reasons for feeding the dried granular hide collagen in parallel with the dry cut or fibered form were to establish some limits, if any exist, to the degree of fiber separation required for gastric and alimentary digestion. The air-dried granules were at the upper limits of size which permit collagen incorporation in food mixtures without adding a gristle texture which is objectionable. The digestibility of these granules at 29% dietary supplementation (Table 2) either with or without inherent fat indicates that collagen need not be reduced to fibers before ingestion. The finding of 90 rather than

Table 2—Rat caloric assay and digestibility of collagen samples compared to gelatin<sup>a</sup>

Supplement	Avg wt gain/rat 7 days (g)	Avg wt change/rat on basal for 2 days (g)	Net change/rat		Urine and fecal output/rat/ 9 days MFB (g)	Apparent digestibility <sup>e</sup> (%)
			Columns 2 + 3 (g)	Minus basal (g)		
(1) Basal control	2.4	0	+2.4	—	4.0	100
(2) Gelatin	20.2 <sup>c</sup>	-3.6	+16.6	+14.2	5.0	93
(3) Collagen <sup>b</sup> (2 mm 10% fat)	19.8 <sup>c</sup>	-4.0	+15.8	+13.4	5.4	90
(4) Collagen <sup>b</sup> (acetone dried 0.060 in. fat free)	15.4 <sup>c</sup>	-2.8	+12.6	+11.4 <sup>d</sup>	5.4	90
(5) Collagen <sup>b</sup> (air dried 0.060 in., 3% fat)	18.0 <sup>c</sup>	-4.2	+13.8	+11.4	5.4	90

<sup>a</sup> 5 Male rats/group, Sprague-Dawley strain, 4 wk of age

<sup>b</sup> Numbers 3, 4, 5 refer to a, b, c in Fig. 1; the 3 collagens and gelatin were fed at a level of 2 g/rat/day as supplements to the basal diet (5 g/rat/day).

<sup>c</sup> Some rats in each group had diarrhea.

<sup>d</sup> Corrected for rejected supplement by 2 rats

<sup>e</sup> Apparent % digestibility = [(supplement intake - increase in urine and feces)/supplement intake] X 100

Example: Collagen (3)  

$$\frac{[14g - (5.4g - 4g)]}{14g} \times 100 = \frac{12.6}{14} \times 100 = 90\%$$

Table 3—Terminal body and organ weights of rats fed a 20% collagen diet for 90 days<sup>a</sup>

No.	Diet Supplement	Sex	Body wt (g)	Organ weights in grams per 100 grams body weight <sup>b</sup>			
				Liver	Kidneys	Spleen	Heart
1	Purina basal	M	477 ± 50	3.47 ± 0.21	0.67 ± 0.07	0.14 ± 0.01	0.29 ± 0.04
2	+20% Casein	M	454 ± 36	3.82 ± 0.39	0.70 ± 0.06	0.15 ± 0.03	0.28 ± 0.02
3	+20% Collagen	M	440 ± 61	3.44 ± 0.27	0.81 ± 0.09*	0.15 ± 0.01	0.31 ± 0.03
4	Purina basal	F	281 ± 17	3.57 ± 0.18	0.67 ± 0.05	0.19 ± 0.01	0.32 ± 0.03
5	+20% Casein	F	295 ± 14	3.33 ± 0.20	0.72 ± 0.02	0.19 ± 0.03	0.35 ± 0.03
6	+20% Collagen	F	280 ± 14	3.52 ± 0.13	0.83 ± 0.05**	0.18 ± 0.02	0.34 ± 0.02

100% digestion of collagen in the rat gut is probably related to the 10–20% unconverted residue usually found in commercial gelatin manufacture.

### Toxicity

The lack of sub-acute toxicity in rats fed diets containing up to 20% collagen for 90 days was not surprising, but confirmation of a long standing assumption. The significantly higher kidney weights in rats fed collagen over those fed casein supplement may be attributable to the higher nitrogen content of the collagen. The kidney weight difference was 12–14%. Collagen is 18.6% N<sub>2</sub>, casein is 15.9%—a difference of 15–17%. A functional hypertrophy of the kidney is known to occur in rats fed high protein diets (Osborne, 1926–1927).

### CONCLUSIONS

DELIMED, washed, fibrous, insoluble hide collagen when fed to rats is well digested (90%) and serves as a source of energy. It is not toxic when fed at a high percentage of the diet for relatively long periods.

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